Chapter

Essay on the Elusive Natural History of Ebola Viruses

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Abstract

This chapter presents a review of what is known about the natural history of the Ebolaviruses in Central and West Africa as well as in the Philippines. All the previous hypotheses on the natural cycle of Ebolavirus are revisited. Also, the main factors driving the virus natural cycle are summarized for the different ecosystems where the Ebolavirus is known to have emerged, including the virus species, the date of emergence, the seasonality, the environmental features, as well as the potential risk and associated factors of emergence. The proposed hypothesis of the Ebolavirus natural cycle prevails an inter-species spillover involving several vertebrate hosts, as well as biotic and abiotic changing environmental factors among other original features of a complex natural cycle. It is also compared with other virus having such type of cycle involving chiropteran as potential reservoir and vector and presenting such original inter-outbreak epidemiological silences. Ultimately, these observations and hypotheses on Ebolavirus natural cycles give some insight into the potential drivers of virus emergence, host co-evolution, and a spatiotemporal dimension of risk leading to identify high risk areas for preventing emerging events and be prepared for an early response.

Keywords: Ebolavirus, bats, chorology, natural cycle, host, one health

1. Introduction

It has been several decades since an unknown fever dramatically emerged, close to the Ebola river, a small tributary of the great Ubangi river in the heart of the Congolese tropical forest of Africa. Since that time, even though the virus responsible for this new hemorrhagic fever has been identified and characterized, the natural history of the eponymic Ebolavirus remains largely unknown. The cradle of the virus remains enigmatic and the emergence of the Ebola fever unsolved. Indeed, the arcane of Ebolavirus natural history is still hypothesized, thanks to an elusive virus that always risen where it was not expected, violent and devastating, and surprising local populations and health systems, as well as the international scientific community. This Ebolavirus eco-epidemiology remains complex while the Ebola fever (alias Ebolavirus Disease) can be considered as an exemplary disease that can be eventually comprehended only with a trans-disciplinary approach that has recently been promoted as a One Health concept. Indeed, it is only when we take into account all disease and virus drivers, including

biotic and abiotic factors of the natural and human environments, that some mechanisms of the Ebolavirus disease emergence, such as spread and circulation, can be ultimately unveiled. For that, we have collected all information available, often estimated, from the time and place of the virus emergence long before the emerging event was identified as it and the epidemic phase was brought to public attention. Moreover, when available we also collect all data on potential natural and accidental hosts, weather and environment chorology, among other multiple factors potentially involved.

Historically, Ebolavirus emerged in Central Africa in the late 1970s, and has re-emerged most recently with the active epidemic (April 2019) in the eastern Democratic Republic of Congo (DRC), by encompassing more than 24 epidemic events from Central to West Africa, to imported infected monkey from Asia to Virginia, and the emerging new Ebola species of the Philippines archipelago [1].

Among the negative sense RNA viruses of the *Filoviridae* family five genera are known, including *Cuevavirus, Ebolavirus, Marburgvirus*, *Thamnovirus*. Among the *Ebolavirus* genus, five Ebolavirus (EBOV) species have been identified [2].

Ebolavirus' (EBOV) first emergence occurred in 1976, as two different EBOV species in two different places in sub Saharan Africa. The Zaire Ebolavirus (ZEBOV) species and the Sudan Ebolavirus (SUDV) were detected concomitantly, a few weeks apart, respectively in the Northeastern Equator province of the Democratic Republic of Congo, DRC (alias Zaire), and in the Bahr el Ghazal province of South Sudan. On the 26th of August 1976 ZEBOV was isolated from missionaries and local villagers of the Yambuku, in the rain forest close to the Ebola river. However, earlier in June 1976, the SUDV had broken out among cotton factory workers in Nzara, Sudan (now in South Sudan) [3].

Then, in 1989, the Reston Ebolavirus species surprisingly (RESTV) emerged in the US (!) and was identified during an outbreak of simian hemorrhagic fever virus in crab-eating macaques from Hazleton Laboratories (now Covance) of Reston county, Virginia. Such primate specimens were found to be recently imported from the Philippines. Then, in 1994 a fourth new species of Ebolavirus was isolated from chimpanzee leaving in the Tai Forest of Côte d'Ivoire and named Côte d'Ivoire ebolavirus (CIEBOV). Finally, in November 2007, a fifth Ebolavirus species, was detected from infected patients in Uganda in the Bundibugyo District and was subsequently identified by the eponymic name of Bundibugyo Ebolavirus [4].

Briefly and extraordinarily among the world of the viruses, the filovirus virion presents a bacilliform (filamentous) shape, like a Rhabdovirus, but presents unique pleomorphic figures with branches and other tortuous shapes. Ebolaviruses have also an unusual and variable long length - up to 805 nanometers (only some plant virus can compete to this filamentous extensive length). However, the internal structure is more classical with a ribonucleoprotein nucleocapsid, a lipid envelope and seven nanometers size spikes. The genome is non-segmented, single stranded RNA of negative polarity with lengths of about 18.9 kb that code for seven proteins, each one having a specific function [5].

Ebolaviruses are known for their high case-fatality rate (CFR) with always less than 2/3 of survivors among the identified cases. ZEBOV, the most frequently isolated Ebolavirus species during the outbreaks, has the highest CFR, up to 90% in some instances, with an average of 83% for the past 37 years. The Uganda BDBV outbreak had a mortality rate of 34%. RESTV imported to the US did not cause disease in exposed human laboratory workers. The scientist performing the necropsies on CIEBOV infected chimpanzees got infected and developed a Dengue-like fever, fully recovered 6 weeks after the infection while treated in Switzerland.

2. When Ebolavirus raised his head in the heart of darkness

Dates and time make History. Indeed, the various reports on the emergence of Ebolavirus in Africa show discrepancies and lack accuracy, for multiple reasons (remote event, reports by different person or team, at different time…) but the only way to forge the history is to label the events with date, time and the environmental factors observed. On July 27, 1976, the first (known) victim to contract Ebolavirus was a cotton factory worker from Nzara, Sudan. Then, in Zaire (DRC) on September 1, 1976, the first Ebolavirus (Zaire ebolavirus, ZEBOV) victim was a teacher who had just returned from a family visit to northern Zaire (6 Jennifer Rosenberg Internet). These two events were the very beginning of the boundless journey of a deadly Ebolavirus outbreaks.

2.1 The Ebolavirus species emerging events

When the virus becomes epidemic in a human population, it does so weeks or months after the emergent event of the virus switching from its silent transmission in a natural cycle to a zoonotic/epidemic manifestation, revealed to the local health system. Let us see in more detail such emerging events of Ebolavirus species (ICTV, 2018) as there were reported or sometime interpreted, in time and place.

Sudan ebolavirus (SEBOV) occurred when the first recorded SUDV broke out among cotton factory workers in Nzara, South Sudan in June 271,976. This was indeed, the first case of Ebolavirus infection recorded and confirmed and also reported as potentially exposed to chiropteran. Indeed, at the Nzara Cotton Manufacturing Factory this first patient was a cloth room worker where bats (mostly *Tadarida* - mops - *trevori*) have a large population in the roof space of their premises. He died in the Nzara hospital on July 6, 1976. Local animals and insects were tested for Ebolavirus without success [6, 7].

Zaire ebolavirus (ZEBOV) was reported in the Mongala district of the Democratic Republic of Congo (DRC; alias Zaire) in August 1976, when a 44-year-old schoolteacher of the Yambuku village, became the first recorded case of Ebolavirus infection in DRC. Also, the schoolteacher travel earlier in August 1976 near the Central African Republic border and along the Ebola River, estimated 90 km NW from the village [6].

Reston ebolavirus (REBOV) had its first emerging event as an imported infected cynomolgus monkey (*Macaca fascicularis*) in October 1989 imported from a facility in the Philippines (Mindanao Island) to Reston, Virginia, USA, where the primate got sick and the virus isolated [8]. In the Philippines, in several instances, the virus was found to infect pigs, in June and September 2008 ill pigs were confirmed to be infested by REBOV (Ecija and Bulacan, Manila island), as well during 2008–2009 epizootics in the island of Luzon (Philippines) [9].

Cote d'Ivoire ebolavirus (CIEBOV) was isolated for the first time, and as an only known appearance, in November 1994, from wild chimpanzees presenting severe internal bleeding of the Taï Forest in Côte d'Ivoire, Africa. A researcher became infected when practicing a necropsy on one of these primates, he developed a dengue syndrome and survived. At that time, many dead chimpanzees were discovered and tested positive for Ebolavirus. However, the source of the virus was believed to be of infected western red colobus monkeys (*Piliocolobus badius*) upon which the chimpanzees preyed [10].

Bundibugyo ebolavirus (BDBV) was then discovered during an outbreak of Ebolavirus in the Bundibugyo District (Bundibugyo and Kikyo townships), on August 1st, 2007, in Western Uganda (Towner et al. [11]). BDBV second emerging

event was observed in the DRC in August 17, 2012 in Isiro, Pawa and Dungu, districts of the Province Orientale [11].

With the exception of REBOV in Philippines and CIEBOV in West Africa, all other EBOVs species emerged in the Central African region. Also, all EBOVs are known to emerged in the tropical rain forest during the inter-season between dry and rainy seasons. Also, REBOV appears to actively circulate in the tropical rain or moist deciduous forest of the Philippines [12].

2.2 From Central Africa to West Africa

2.2.1 Concurrent emergences of Ebolaviruses

On several occasions, concurrent emerging events of Ebolavirus have been observed. Indeed, such events occurred in places geographically distant, independent, and unconnected. The Ebolavirus was isolated and the strains different, even they belonged to the same species of Ebolavirus, altogether in favor of a different origin from an elusive natural reservoir, thus eliminating the notion of leaping from one site to the other. In that matter, the following observations are a paradigm: From its inceptive emergence the Ebolavirus was identified in Sudan at the cotton factory and a few days later at Yambuku, Zaire. The Ebola Sudan and Ebola Zaire viruses emerged concurrently in 1976 in the Congo basin of Central Africa; More than 20 years later the virus emerged and reemergence from 1994 to 1996 in a different places in Gabon, in a successive and timely overlapping events but in unconnected areas from where different strains of the same EBOVZ were isolated [13]; More recently, during the 2014–2016 dramatic Ebolavirus disease (EVD) emergence of in West Africa where the virus emerged in late December 2013 of a 18-month-old boy from the small village of Meliandou (Guéckédou district, South-Eastern Guinea) believed to have been infected by bats [14], concurrently, in August 2013, the Ebolavirus reemerged in the Equator province of DRC - different places and different strain of ZEBOV [15].

It is remarkable that most of these emerging events occurred during or close to the end of the rainy season which generally stretches from August to October in the domain of the Congo basin tropical rain forest.

Altogether, these observations are in favor of environmental factors of emergence favoring, when they occur synchronously in the same place, the spillover of the virus from its hidden natural cycle to an accidental and susceptible host. Therefore, these plural and concomitant emerging events play against the theory of Ebola virus diffusing in oil spot in Central Africa [16]. This original pattern of concurrent emergences could explain also the relative stability of the virus strains which remain for years in the same environment, and the interepidemic silences which require several fundamentals (i.e. concurrent risk factors) to be broken.

2.2.2 An unexpected broader domain of Ebolavirus circulation

The first evidence that showed that Ebola virus had previously circulated in areas without any known cases of disease came in 1977, near the Ebola outbreak in Tandala, DRC, just 200 miles west of the first known cases in 1976 [17]. Blood samples obtained from individuals in areas with no previous symptoms of Ebola were found to contain antibodies for Ebolavirus, indicating a previous or ongoing infection with that virus. Because subclinical illness is always a possibility with viral infections, the presence of these Ebolavirus-specific antibodies could only be explained by exposure to the virus, which is somewhat reasonable in an area that is

endemic to the disease. But how do we know the true endemic zone of a virus such as Ebolavirus?

Endemic zones are primarily based on where disease can most likely be expected, and are determined by historical accounts of disease, as well as supplemental information such as where animals or insects that might transmit the disease are located. With respect to the Ebola virus, outbreaks that occur in Central Africa, in or near the Congo River Basin, are expected; outbreaks that take place elsewhere are unexpected and can be problematic, as was the case for the 2014–2016 West African outbreak. And yet, scientists have highlighted the presence of Ebola antibodies well outside the endemic zone for disease for decades.

In the early 1980's, research based at the Pasteur Institute in Bangui, Central African Republic, demonstrated for the first time that the population of central Africa presented natural antibodies against the Ebolavirus strains of Zaire and Sudan [3, 4]. Research also showed for the first time that several mammal species had Ebolavirus-reacting antibodies, including rodents, dogs, and others. Initially, the scientific community was skeptical of the findings, due to the type of antibody tests used, and because the prevalence of these antibodies was unbelievably dispersed and at a high level of prevalence. However, a 1989 follow-up study confirmed methodology and preliminary observations, and expanded the results to include similar observations in Cameroon, Chad, Gabon, and Republic of Congo (the latter two of these countries would have their first Ebola outbreaks in 1994 and 2001, respectively) [5]. Moreover, such Ebolavirus antibody prevalence was found in West Africa (e.g. Senegal, Chad, Sierra Leone), preceding the catastrophic 2014–2016 Ebolavirus outbreak [18]. Subsequent studies have determined that 20–25% of persons living in or near the Congolese rain forest are seropositive for Ebola, despite never exhibiting symptoms [19].

Today, Ebola antibody prevalence is widely distributed across the African continent in the absence of severe clinical presentation and/or outbreak manifestation. A 1989 study even found Ebola Zaire antibodies among people living in Madagascar, an island country that has never had a single known case of Ebola, and which has been geographically separated from continental Africa for 100 million years [20].

Risk mapping, including ecological and geographical distribution <10-13 cm/s first hour, and extended, highly sensitive and specific environmental and biogeographical models based on EBOVs susceptible mammalian biogeography in Africa, show a robust and precise potential distribution of EBOVs in Africa that clearly overlap the African tropical rain forest biome of the Guinea-Congo forests (including the Congo basin rain forest, and the Occidental relic of the Congolese rain forest spreading from Guinea to Ghana) and the southern band of the Sudan-Guinea Savanna [21].

Also, as a result of potential Ebolavirus (or Ebolavirus antigen) exposure, serological markers have been found in vertebrates outside of Africa. With the exception of Philippines, where REBOV is known to circulate in monkeys and pigs, thus showing its ability to infect multiple animal species, in several instances serological evidence of Ebolavirus exposure has been detected in many vertebrates, particularly chiropterans [9]. Definitely, bat populations in Bangladesh and China present antibodies against ZEBOV and REBOV proteins [22, 23]. Ultimately, it appears that EBOVs are widely distributed throughout Africa, West and Central, and Asia. Moreover, risk mapping of filovirus ecologic niches suggests potential areas of EBOVs distribution in Southeast Asia [24].

The unexpected detection of REBOV first in Virginia, for the reason we know, and then the astonishing discovery of its circulation and natural cycle in the Philippines gave a rethinking of the entire family of Ebola viruses previously known mainly on the African continent [25].

From these observation and facts, the potential circulation of EBOVs in its natural cycle appears much wider than expected, while the emerging events we can witness appears to be only a tip of the iceberg in the wide Congolese tropical rain forest.

2.3 From the index case to the epidemic chain, outbreak, and pandemic

The fundamentals of emergence are changing in the heart of the rainforest and elsewhere: changing times, when the means of transmission switch from foot to motorbike, when knowledge conveyance has switched from paper reporting to the internet.

Let us examine the risk of expansion for Ebolavirus. Indeed, the factors of transmission of the virus to man and man to man are essential to take into account in this context. Moreover, it is extremely important to note that these factors are subject to permanent changes in societies whose trade and means of communication are drastically changing as a result of health systems, responses and preparedness for epidemics at national and international levels, policies, and the economy.

So, with the experience gained for more than 40 years, the strategies of struggle are clearly defined, but the societal changes that are taking place make their application difficult and sometimes impossible (e.g., the 2019 outbreak in the DRC, where political institutions have prevented an adapted response). Situation and the epidemic are perpetuated.

There is also a growing means of communication, both smartphones and motorized transport, to travel more quickly as ever, between the epidemic zone of EVD and the family [26].

Thus, during the emergence of the Ebola virus in West Africa, all of this means of communication played a fundamental role in the regional spread of the epidemic, until it became a pandemic risk when the virus was exported to other countries of the African continent and, outside Africa in Europe and North America [27].

3. A strange iteration of epidemic events with unexplained virus disappearance

It is known for several other transmitted viruses that during the inter-epidemic silences several factors can be responsible. In general mass herd immunity (natural of due to acquired immunization i.e. vaccine) of the permissive hosts force the virus in its natural cycle without apparent clinical manifestation in the hosts (e.g. Most by the arbovirus classically yellow fever, Dengue, Japanese encephalitis, West Nile, Zika etc.).

The *Paramyxoviridae* and *Rhabdoviridae* are the two other viral families in the order Mononegavirales, genetically closely related to the Filoviridae and having chiropteran as reservoir and/or vector [28]. Indeed, it is interesting to note that megachiropteran fruit bats are reservoirs of Hendra and Nipah viruses of the Paramyxoviridae family [29]. When, Microchiroptera bats are the probable ancestors of all rabies virus variants of the Lyssavirus genus in the family Rhabdoviridae and infecting presently terrestrial mammals [30]. Both also present this cryptic interepidemic silences that has not been yet clearly understood. The Nipah emerged one time in Malaysia (1999), thought to have its original cycle in PNG, and ultimately reemerged more than 3500 km away in Bangladesh in 2001. From its inception, again the Marburgvirus (the closest to EBOVs in the family of Filovirus), emerging events from an expected natural foci occurred within the path of time

including 4 to 11 years of inter-epidemic silences occurring mostly in distant sites of Eastern and South Africa (Uganda, Zimbabwe, Angola, Kenya).

If one were to describe the history of Ebola outbreaks, one could simply construct a timeline, with a point on the line for each outbreak. You could create this timeline with a varying number of points, depending on your methodology, but regardless of how you built your timeline, there would be spaces between these points. This is due to the nature of Ebola; it appears, it disappears, and it appears again. To the Ebola virus, these gaps are periods of convalescence. To us, they are periods of absence and mystery, and one of these gaps stands out as the most mysterious (**Figure 1**).

The CDC lists five Ebola outbreaks in the late 1970's. The "first" Ebola outbreak took place in 1976, though we now recognize the event as two simultaneous and separate outbreaks. Between June and November 1976, 284 cases (151 deaths) of Ebola Sudan occurred near what is now Nzara, South Sudan; between September and October 1976, 318 cases (280 deaths) of Ebola Zaire occurred near what is now Yambuku, Democratic Republic of Congo (DRC). In November 1976, a researcher in England that was working with samples from the Nzara outbreak accidentally infected himself; CDC lists this accident as the third Ebola outbreak (the individual recovered). In June 1977, a child became sick and died from Ebola Zaire in Tandala, DRC though there was only one confirmed case, subsequent epidemiological investigations of the area uncovered several other historical, probable cases. Finally, between July and October 1979, 34 cases (22 deaths) of Ebola Sudan occurred, unbelievably, in Nzara, Sudan – the same community where the first cases of Ebola emerged just 3 years prior. In the span of just 39 months, the terror of Ebola had introduced itself to the world five times (638 cases, 454 deaths) and then… silence.

Ebola would not reappear for 10 whole years, and even then, the subtype was Ebola Reston, which we now know does not affect humans. Though CDC lists four Ebola Reston outbreaks between 1989 and 1992, the world would not see another case of Ebola virus disease in humans until late-1994, in Gabon. Even then, the outbreak (52 cases, 31 deaths) was mischaracterized as yellow fever for several months. Perhaps the virus's long absence from the spotlight had removed it from the collective consciousness in 1994, certainly in the presence of those pathogens that had been circulating and consuming our attention in the meantime.

This fifteen-year disappearance of Ebola, particularly in light of its frequent and severe outbreaks in the late 1970's, has perplexed researchers for decades.

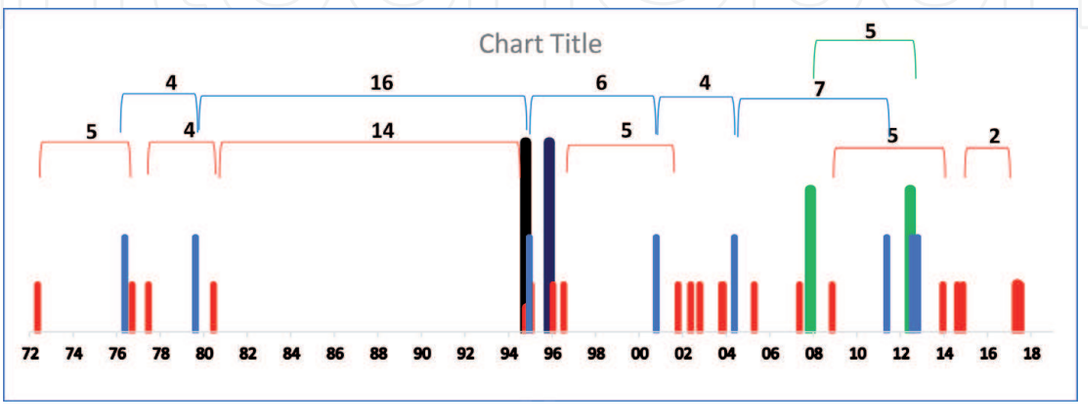

Figure 1.
Timeline of Ebolavirus emergence. Emerging events (bars) red = EBOV; blue = SEBOV; green = BDBV; horizontal axis = years 1972–2018; vertical axis = no value. Numbers above brackets = years of silent inter-emerging event.

The mystery lay, to some extent, within the lack of complete knowledge of the virus reservoir, though scientists are now having their long-held suspicions in bats confirmed. It's hard to detect disease when you cannot pinpoint the source. Surveillance and reporting have been another confounding element. How many times in that fifteen-year period was an illness misdiagnosed as yellow fever, dengue hemorrhagic fever, or some other similar illness, because of lack of knowledge or diagnostic capabilities, or simply because there was no health care around? We will probably never be able to answer this question. Finally, our perceived zone of endemicity at the time was limited to northern DRC and southern Sudan. Was the virus appearing elsewhere, unbeknownst to us? We certainly were not expecting it to emerge in Gabon in 1994, and Uganda in 2000, and West Africa in 2014 [31].

Scientists today continue to be perplexed by the emergence of the virus. What brings Ebola out from its hiding place? Is its emergence/re-emergence tied to climate change? globalization? the changing interface between humans and wildlife? If it has to do with any of these increasingly significant factors, how do they explain the fifteen-year disappearance?

These days, the virus comes and goes with some predictability—since 2000, outbreaks have approached a near-annual incidence, sometimes skipping a year, sometimes lasting more than a year. The periods between outbreaks are growing shorter. Is this because our capability to detect Ebola outbreaks is improving, or is the virus able to infect humans more frequently? One thing is for sure: the world knows that when one outbreak ends, another will eventually follow, and we need not wait 15 years.

4. Toward the discovery of the natural cycle of the Ebolaviruses

4.1 The discovery of a putative natural reservoir of Ebolavirus

Since the ZEBOV and SEBOV emergence, extended field studies have been conducted to discover the reservoir of EBOVs [32] including the 1976 first recorded DRC outbreaks and Sudan, the 1979 outbreak in DRC in 1979 and 1995 following the Kikwit outbreak, the same year in the Tai Forest and in 1999 in the Central African Republic [33–38] . A total of more than 7000 vertebrates and 30,000 invertebrates were sampled and tested for the presence of EBOVs. Limited finding was inconclusive for an potential EBOVs reservoir status among all these animals. Moreover, while several animal species (Bats, birds, reptiles, mollusks, arthropods, and plants) were experimentally infected with ZEBOV, only two fruit bat species (*Epomophorus* spp. and *Tadarida* spp.) developed a subclinical transient viremia [39]. If these results were not confirmed in the natural settings, they indicated the potential for chiropteran to be natural for EBOVs [40].

Also, historically, the first documented case of EVD in Sudan in 1976, the index case was located (by the World Health Organization) in a cotton factory far from the forest block, where the only wild significantly abundant species was an insectivorous bat species [21].

Since the discovery of EBOV in 1976, more than half of the epidemic outbreaks caused by EBOVs have broken down between Gabon and the DRC. Following the successive EBOV outbreaks in Gabon from 1995 to 2001 affecting several animal species non-human primates, and wild ungulates and responsible of the dramatic decline of great apes (gorilla and chimpanzee) populations in the region (Leroy et al. [16]), researchers engaged several missions of captures of wild animals in the forest areas affected by the recent past epidemics. Also, 1030 animals were captured and analyzed, only three species of fruit bats were found infected with the ZEBOV by PCR including: *Hypsignathus monstrosus*; *Epomops franqueti;* and *Myonycteris*

torquata. Moreover, antibody reacting anti-Ebola were detected in these species as well as for the genus *Myonycteris* spp. leading ultimately to design Chiropteran as a potential reservoir of EBOVs [41].

Since then, many studies have converged in favor of the role of chiropters in maintaining EBOV in the wild (Caron et al. [42], Leendertz). In addition, a recent study of bats in Sierra Leone showed the association of an EBOV like with several species of bats (*Mops condylurus* and *Chaerephon pumilus*) from the Molossus family [43]. Moreover, a potential direct exposure to Ebola infected fruit bats was also reported as a putative index case of large epidemics [44, 45]. Moreover, further studies reported on direct infection of natural hosts (primates) by EBOV infected bats as highly plausible, given that bats, especially fruit bats, are frequently hunted and consumed as bushmeat by human when *Cercopithecus* species hunt roosting bats for consumption [46] also preying on bats has been reported in *Cercopithecus ascanius* and *C. mitis* (East Africa) as well as bonobos (DRC) [47]. It is also possible that different modes of exposure to Ebola virus could lead to different antibody profiles, that is, contaminated fruit vs. contact with infected bats during hunting [44, 47, 48].

Altogether, several fruit bats (*Epomophorus wahlbergi*) and insectivorous bats (*Chaerephon pumilus, Mops condylurus*) experimentally survive to EBOV infections [39], EBOV RNA and/or anti EBOV reacting antibodies were detected also in several other fruit bat species (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata*, *Eidolon helvum, Epomophorus gambianus, Micropteropus pusillus, Mops condylurus, Rousettus aegyptiacus, Rousettus leschenaultia*) giving more insight of the potential for chiropteran to be a potential host or reservoir host of EBOVs [22, 49, 50].

Interestingly, REBOV was also found associated with the bats in its natural habitat of the Philippines [51]. Also, again in this same *Filoviridae* family, Marburg viruses in Africa are clearly associated with bats [32, 52] as well as the Cueva virus in Europe [53]. While REBOV has been find associated with fruit bats, *Roussetus* spp. (Pteropodid family), each filovirus genus is associated with a specific chiropteran group including: Marburgvirus with a specific fruit bat, *Roussetus aegyptiacus* (Pteropodid family); and Cuevavirus with insectivorous bat, *Miniopterus schreibersii* (Miniopterid family); except for *Thamnovirus* isolated form fresh water fish.

Moreover, several virus groups are known to hold bat-borne viruses including the coronaviruses, hantaviruses, lyssaviruses, lassa virus, Henipavirus, filovirus which are among the most severe of the emerging viruses [54, 55].

Conclusively, this was the first evidence of chiropteran as a potential reservoir and/or vector of EBOV, while several wild animals, in particular great apes were find highly sensitive to EBOV infection. Also, if several species of chiropteran have been identified as a potential virus reservoir,

4.2 The most complete figure of a putative Ebolavirus natural cycle in the central African raining forest

From all above observations, records and historical events of EBOVs emerging events, several fundamentals of emergence have been identified as well putative time and space of such events where, that is when the virus jump from the cryptic natural cycle of the reservoir-vector to manifest itself clearly as an open index case of infection in a susceptible host and the potential opening epizootic or epidemic chain.

4.2.1 The actors

Again, from the literature numerous vertebrates appears to be permissive to infection by EBOVs, however, due to their ethology, including environmental habits, societal structure, density and their ability of intra and interspecies to mingle.

Altogether primates appear highly susceptible to EBOVs infection including non-human primate apes, gorilla and chimpanzee, but also cercopithecids (e.g. colobus) but also small wild ungulates (e.g. forest duikers) and eventually domestic animals (e.g. dogs) [32, 56–58].

One can summarize that EBOVs natural hosts belongs to chiropteran as a potential host reservoir represented mostly by Pteropodidae in Africa (REBOV and Roussetus; Bombali virus and Molossidae), and as secondary natural or accidental wild and domestic hosts including several other mammals: primates (Colobus, Cercopithecus), non-human primates (Gorilla, chimpanzee), wild ungulates (duikers) and, human primates. Also this needs to be taken into account with respect to other permissive species to EBOVs, indeed, as an example, if Roussetus spp. was shown to carry EBOVs reacting antibodies more recently *R. aegyptiacus* bats were demonstrated to unlikely able to maintain and perpetuate EBOV in nature while the natural transmission of filovirus in *R. aegyptiacus*, resulting viral replication and shedding are unknown [59].

4.2.2 The stages

The African Rain forest of the Congolese basin appears to be the epicenter of EBOVs emerging events. More than 80% of the emerging events of EBOVs occurred in the Tropical zone under the influence of the (Intertropical converging zone, ITCZ) from five degree North to 5 degrees south and oscillating as much as 40 to 45° of latitude north or south of the equator based on the pattern of land and ocean beneath it [28] (**Figure 2**).

Temperature and precipitation data for Africa (average data computed from 1960 to 1990, 300 m resolution [HIJ 05]) were integrated with the distribution map of the emergent events of the Ebola virus and the values calculated for each of the emergence points [60].

On all emergence points, the temperature at the time of emergence is not significantly different from the average annual temperature over 30 years. The difference in temperature between the moment of emergence and the average temperature (of 30 years monthly average) of the hottest month does not show any difference either. Emergence would not be directly related to temperature.

When we compare Ebolavirus emerging events time and the rainfall, there is strict quantitative correlation between rainfall and emergence: Most of the emergent events (93.8%) occurred during the rainy season (**Figure 2**). For precipitation values, there is a slightly statistically significant ($p = 0.02$) positive difference between the average precipitation of the month of emergence and the average of the monthly average precipitation (over 30 years), indicating that precipitations are higher when emergences occur. There is an even more statistically significant ($p = 0.003$) positive difference when considering precipitation of the month preceding the emergence. Emergence is therefore likely to be associated with rainfall intensity and the rainy season. 10/32 emergences occur at the beginning of the rainy season, 9/32 in the middle, and 11/32 at the end. Only 2/32 emergences occurred in the dry season.

When referring to land use (**Figure 3**) the temperature at the 6 emergence points in "Cropland" is highly significantly less ($p = 0.005$) than 15% (21.6°C) at temperature (24.4°C) to the 9 points in "Tree cover, broadleaved, evergreen, closed to open", however the average temperature of the Cropland (21.6°) is to a degree less, significantly lower ($p = 0.01$) than that of the "Tree cover" (24.5°C).

Ultimately, taking into account these environmental factors, when we look for an association between the emergent events of the Ebola virus and the

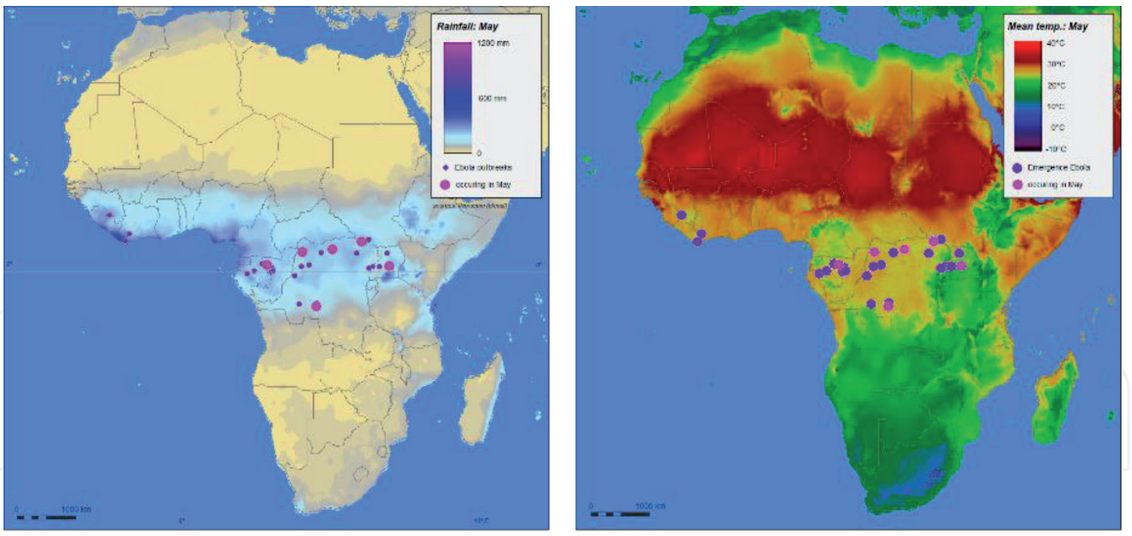

Figure 2.
Emerging events of Ebolavirus and climate since the Ebola fever inception in Africa. Left = annual rainfall; right = annual temperature. To illustrate the association temperature/rainfall and emergence, the month of May was chosen because it is at this time of the year that we observe the most emergent events of the Ebola virus. Temperature and rainfall are expressed as an annual average for the period under consideration. The precise location of 32 Ebola emergent events are here integrated into the global climatic map of Africa. Only 30-year average values per month of rainfall are available for the study period (ref.: WorldClim world databases) as well for the average monthly temperature.

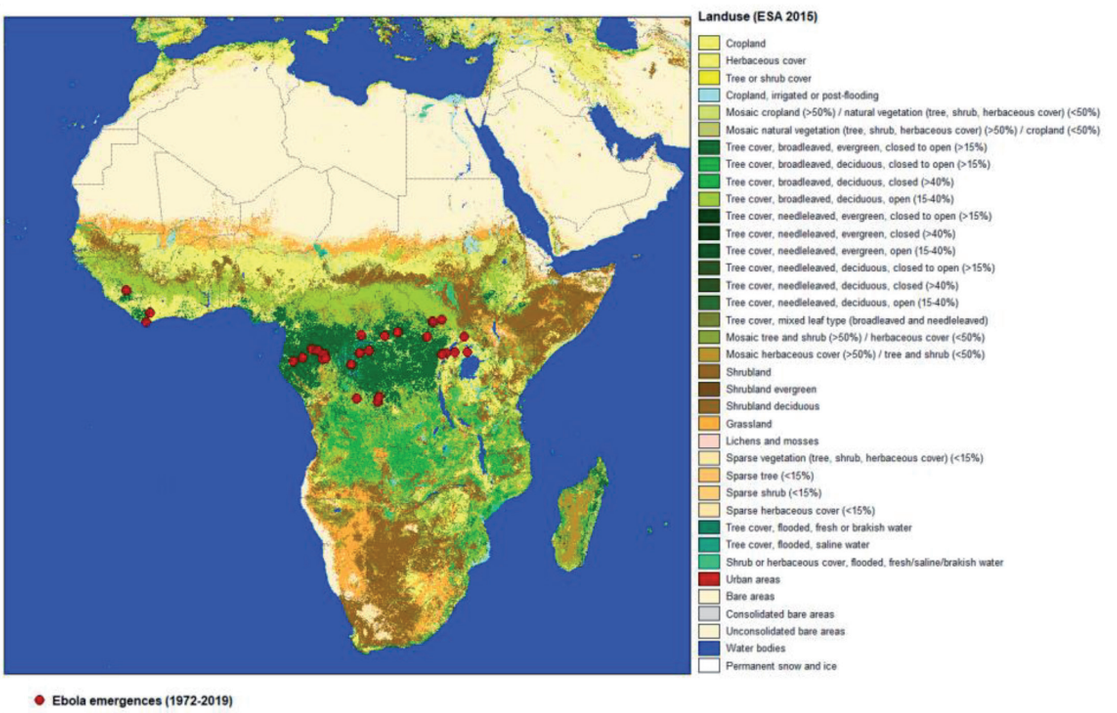

Figure 3.
Environmental factors surrounding Ebolavirus emerging event: Land use and places of Ebola virus emergence in Africa from 1976 to 2014. Land use from ESA 2015, 300 m resolution; red circle = putative place of the Ebola virus emergence (index case). Estimated Ebola emergence places are superimposed on the land use layer. The identification of the land use types were 32 points (red circle) representing the putative places of Ebolavirus emergence are superimposed and are distributed as follows: (1) cropland: 6, (2) herbaceous cover: 5, (3) cropland mosaic: 5 (> 50% natural vegetation vs. <50% tree, shrub, herbaceous cover), (4) tree cover with: (a) 15% of broadleaved, evergreen, closed to open: 9, (b) 15–40% of broadleaved, deciduous, open: 2, (5) flooded, fresh or brackish water: 1, (6) urban areas: 3, and (7) water bodies: 1. The limitations of this interpretation are linked to the accuracy of the location of Ebolavirus emergence sites (from literature and reports) and, to the evolution of vegetation cover over the past decades since the first emergence of the Ebolavirus occurred in Africa.

characteristics of the places of these emergences (i.e. land use, temperature, rainfall) it turns out that the emergences are always in the zone of heavy rainfall, but nevertheless do not follow the moving of the rainy season. Moreover, these emergences remain always and remarkably close enough to the Equator, therefore in the equatorial forest area with a high hygrometry, and a moderate annual temperature. However, the temperature at the time of emergence is not significantly different from the average annual temperature (at the points of emergence) which does not allow to distinguish seasonal effect in the emergence-temperature relationship. Conclusively, we did not identify a seasonality associated with the time of emergence, however the emerging events occur in specific geographic zone characterized by several environmental factors. Finally, the emergence zones are in areas of Land Use with specific temperatures not related to seasonality. Ultimately, it is also remarkable that all these emerging events occurred in an area with a highly potential presence of apes, virus-sensitive hosts.

4.2.3 Fundamentals and domains of emergence: a theory for a natural cycle of EBOVs in Africa

Also, the EBOVs species are closely genetically related, their seems to occur by foci in nature. The host appears to be the same, natural or accidental, and the transmission done by direct contact with infected hosts or its biological products [50, 61]. Altogether, in the early 2000s, before the identification of chiropteran as a potential host-reservoir of the EBOVs, a hypothetic natural cycle was described empirically based on seasonal environmental climatic factors [55]. Then, taking into account bats as a potential reservoir-host, the question of virus transmission was central to consider while environmental factors appears to play a major role to the host and their natural cycle (Chiropteran physiology) (climate/fructification, chorology, bats physiology). Several factors of emergence were then listed including: Chronic infection, infected organs, virus shedding, close encounters between reservoir and susceptible hosts, food and water resource, seasonality, chorology (i.e. causal effect between geographical phenomena – season) in the tropical rain forest and the spatial distribution of chiropteran (i.e. index site of Ebola emerging events).

Epidemiological field surveys indicate that mass mortalities of apes and monkey species due to Ebola virus often appear at the end of the dry season, a period when food resources are scarce. Restricted access to a limited number of fruit-bearing trees can lead to spatiotemporal clustering of diverse species of frugivorous animals, such as bats, nonhuman primates, and other terrestrial species foraging on fallen partially eaten (by bats) fruits. These aggregates of wild animal species favor the contact between infected and susceptible individuals and promote virus transmission. The dry season aggregation of reservoir host species involved in natural maintenance cycles, augmented by incidentally infected secondary hosts serving as sources for intra- and interspecific transmission chains independent of repeated spillover from the reservoir host, provides an ecological setting for amplifying enzootic transmission of Ebola virus when a vertebrate hosts are concentrated around a scarce number of water sources [62].

In addition to this dietary impoverishment, there are behavioral and physiological events occurring among bats during the tropical dry favor the contact frequency and intimacy between bats, which can promote transmission of Ebola virus to others and increase R0. As an example, megachiropteran fruit bats breeding activities and intraspecific competitions between males and grouped *kidding* of females favor the contact between individuals. Moreover, pregnancy can involve physiological changes among female bats that alter immune functions and eventually favor virus

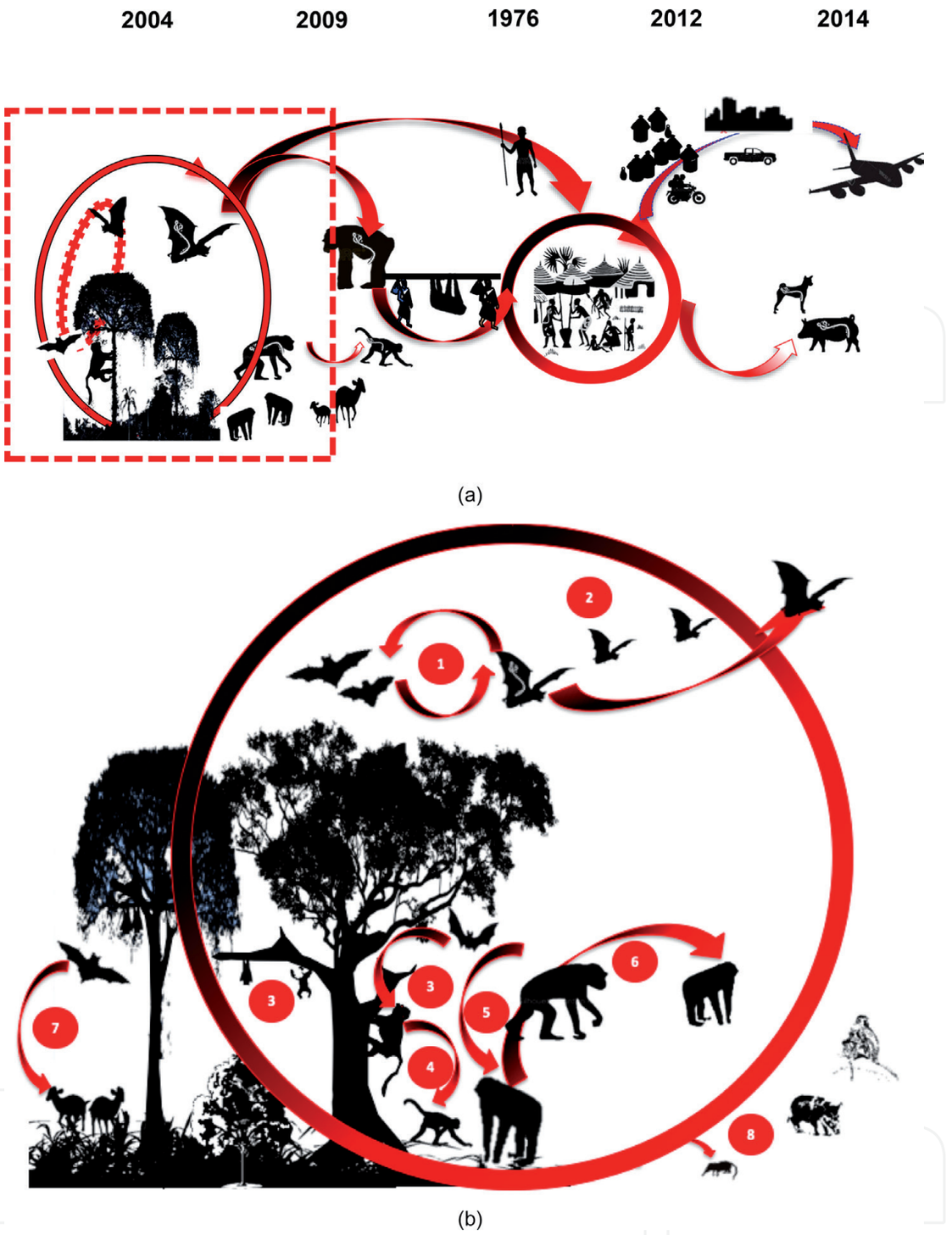

Figure 4.
(A) Understanding Ebolavirus enzootic and epidemics. Red arrows = cycles of transmission; dashed square = a putative natural cycle of Ebolavirus in Central Africa (see B). Fruit bats are considered to be a putative reservoir of Ebola virus in Central Africa after 2004; In 2009, several non-human primate epizootic are reported; 1976 was the first emerging events and subsequent epidemic chains in remote area of the rain forest and close by; 2012 showed a dramatic spread of the virus associated with motorized transportation and ground network; In 2014 urban epidemics are reported as well as a pandemic risk and become an international public health emergency. (B) Putative natural cycle of Ebolavirus in Central Africa. Red arrow indicates Ebolavirus transmission. Numbered red circle of transmission: (1) sylvatic inter- and intra-species transmission; (2) chiropteran migration; (3) chiropter to primate (close contact of dejection); (4) primate inter species (Cercopithecus/chimpanzee); (5) primate to primate (non-human primates); (6) non-human primate epizootic (gorillas); (7) chiropter to duikers; and (8) consumption of chiropteran infected food by shrew or wild pig.

shedding. Parturition among the African megachiropteran bats occurs throughout the year, although seasonal peaks provide birthing fluids, blood, and placental tissues, potentially Ebolavirus infected, falling on the ground as a medium highly

attractive and readily available to scavenging terrestrial mammals [50, 56, 63] (**Figure 4A** and **B**).

5. If we had to conclude

Based on historical data and observations, the presented hypothesis of the natural cycle of Ebolavirus emergence prevail an inter-species spillover as the complex natural cycle involving several hosts (reservoir, vector, amplifier), as well as biotic and abiotic factors in a changing environment among other original features.

Although the natural cycle of EBOVs remains in the darkness of the rain forest, strong findings and comparative analysis of close parents of the filovirus throw some light to a potential natural cycle of EBOVs in Africa. EBOVs clearly appear linked to chiropteran and dependent for merging events in the environmental factors. Indeed, it appears that filoviridae are often associated with chiropteran while the emergence of the virus strains occurs as a sparse focus with a silent period of cryptic virus circulation. When virus transmission, i.e. spillover, from a hidden natural cycle, to accidental hosts occurs, it happened in a specific time-frame often linked to the season.

One can retain is that the EBOVs complex natural cycle is yet not on entirely elucidated and certainly dependent on environmental factors – associated with a specific environment of the chiropteran species incriminated (i.e. Different territories, different cycle) - leading to multiple, sometime concurrent, temporally and timely emergence in focus.

Although, other hypothesis has been suggested elsewhere including the Ebola virus Disease as an arthropod borne disease among others [42], there is important fundamental matters to consider as well before providing more.

However, beyond these hypotheses, fundamental questions subsist in order to go further learn. We can cite in particular the mystery of kin between the Reston virus of Asia and the Ebola viruses of Africa, would there not be a missing link in a geographic area yet to discover. Do the filovirus exist in the Americas hidden in the darkness of the tropical forest? Also, the Ebolavirus seems genetically stable, related to particular species of chiropter, was it to think about a co-evolution of the host and the virus in this closed environment of the forest of the tropical? Today, with the endless epidemic unfolding in the DRC, should we revisit our tools and strategy of struggle in an ever-changing world? [64].

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Conflict of interest

All authors do not have any conflict of interest whatsoever with this published manuscript.

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